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Distribution range of Montane Crab, *Sundathelphusa tenebrosa* Holthius (Decapoda: Brachyura: Parathelphusidae) in Borneo

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Abstract

A montane crab, *Sundathelphusa tenebrosa* was first described by L.B. Holthius, a well-known carcinologist in 1979, based on a male specimen collected from a clear and cool mountain stream near Camp 2 at west ridge of Gunung Mulu National Park (GMNP). Since then, no specimen had been collected from other places in Borneo. Due to very restrictive distribution, *S. tenebrosa* had been categorized as endemic to Gunung Mulu National Park (Ng and Yeo, 2007). Its single coeneger, *S. aspera* is only found in Sabah.

However, current surveys in Pulong Tau National Park (PTNP) Miri and Murum River system in Belaga have collected a number of specimens of *S. tenebrosa*. Eleven specimens (8♂, 3♀) from six streams in PTNP, and seven specimens (6♂,1♀) from three tributaries of Murum River were examined, and all of them agree very well with the type descriptions and pictures by Holthius (1979). The most conspicuous character of *S. tenebrosa* is the presence of very deep cervical groove that is clearly visible throughout the carapace length (Plate 1). The male has T-shape abdomen and the first gonopod is short. With this finding, *S. tenebrosa* is no longer endemic to Gunung Mulu National Park.

Another important finding is the presence of this species in acidic habitats. Unlike streams in GMNP

Plate 1: *Sundathelphusa tenebrosa* (♂) from Lawan River, PTNP. Arrow indicates the cervical groove.

which are alkaline, most of the streams in PTNP are acidic because of kerangas forest in the catchment areas. The occurrence of this crab in both water types indicates that its distribution may not restricted by water acidity.

For comparison, no single specimen of *S. tenebrosa* had been collected from 19 streams in Bau limestone areas (Ng & Jongkar, 2004). Compared to Bau limestone area which is low-lying limestone forest, the GMNP, PTNP, and Murum River system are located at relatively high elevation. To testify whether distribution of this species is related to altitude, more studies have to be done to cover more areas. This species however, seems to be confined to the northern region of Sarawak.

Sundathelphusa tenebrosa is categorized as endangered species (Ng & Yeo, 2007). Besides Gunung Mulu and Pulong Tau National Parks, the habitats of this species in Murum River system had been heavily disturbed by logging activities. Therefore, information on the distribution range of *S. tenebrosa* is highly important for conservation and habitat protection of this species.

Acknowledgement

This work was supported by ITTO Project Transboundary Biodiversity Conservation - Pulong Tau National Park PD 224/03 Rev.1 (F), and Murum Hydroelectric Dam Project NREB/F/00093. Our sincere thanks are addressed to Dr Paul Chai (ITTO Project Manager), Dr Penguang Manggil and Haji Sapuan Ahmad (ITTO Projects Coordinators), study team members and many other people who have in one or another contributed to the success of this work.

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Plate 2: Typical habitat of *Sundathelphusa tenebrosa*; Small stream (usually 1st order) with gravel and pebbles bottom substrates, shaded, cool and fast flowing water.

The Diversity and Distribution of Beetles (O: Coleoptera) at Kubah National Park, Sarawak.

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A total of 123 species (Fig.3) representing 18 families (Fig.4) were collected from Kubah National Park, Sarawak between July, 2006 and March, 2007. The majority of them belonged to the family Scarabaeidae (45 species), followed by Chrysomelidae (18 species).

The rest were relatively low in number, with less than 10 species. The Families Brentidae, Cantharidae, Rhipiceridae and Staphylinidae were each represented by one species only. The number of species for each family is shown in Fig. (3) below. Overall, species diversity of beetles at Kubah National park is high both in term of species evenness and species richness.

In terms of individuals 609 beetles were collected within twelve sampling days from 26 August 2006 until 1 September and from 1 December 2006 until 8 December 2006. (Fig. 2). Scarabaeidae with 339 individuals is the most represented family included *Onthophagus diabolicus* (38 individuals), which were found in abundance in the ground of study areas. Second is Carabidae (109 individuals) followed by Chrysomelidae (37 individuals).

The most abundant species at Kubah National Park, particularly Mount. Serapi, is *Colpodes fryibates* (Carabidae) (Fig.1) with 100 individuals, followed by *Onthophagus diabolicus* (Fig.2) (67 individuals). Other species in this family have very low individual numbers (between 1 to 3 individuals).

Figure 1. *Colpodes fyibates.*

Figure 2. *Onthophagus diabolicus.*

Six Scarab species were found to be very common to Kubah. They can be found in large numbers scattered within the park. They are SP29, SP24, *Onthophagu diabolicus*, SP27, *Anomala matriculate* and *Onthophagus aurifex* with 67, 50, 38, 31 24 and 24 individuals respectively.

The distribution of beetles at Kubah National Park varies with location and elevation; more beetles were encountered at the lower elevation of Mount Serapi. However, high numbers of *C. fryibates* and most species of Scarabs were encountered at all the three sampling sites.

Out of 123 species, 72 were found at the lower elevation of Kubah hill. They belong to 13 families. Most of them were Scarabs with 28 species followed by Chrysomelids and Tenebrionids with 9 and 7 species each. At the middle elevation, 17 species comprising of 5 families were recorded. The Chrysomelids recorded the high of number of species (15 species) followed by Scarabaeids (8 species). In the upper elevation, 25 species from 11 families were captured with Scarabaeids recorded as having the highest number of species (16 species). The rest were represented by single individuals.

Five families may be considered rare and were found in one particular elevation and had very low number of species and individuals. One species of Staphylinidae is found in the lower elevation while Brenthidae and Cantharidae were found in the upper elevation with also 1 species each. Both species of Buprestidae were found in the middle elevation while one species of Lanthridiidae found at both the lower and upper elevations.

The *Onthophagus* spp. was detected only at the lower and middle elevations of Mont Serapi with 78 individuals collected. SP29 was well distributed in height of over than 250m above sea level and were generally found in the middle to upper elevations. Sixty seven (67) individuals were captured from both elevations. This indicated that ground beetles were more diverse in the middle elevation compared to the other two levels.

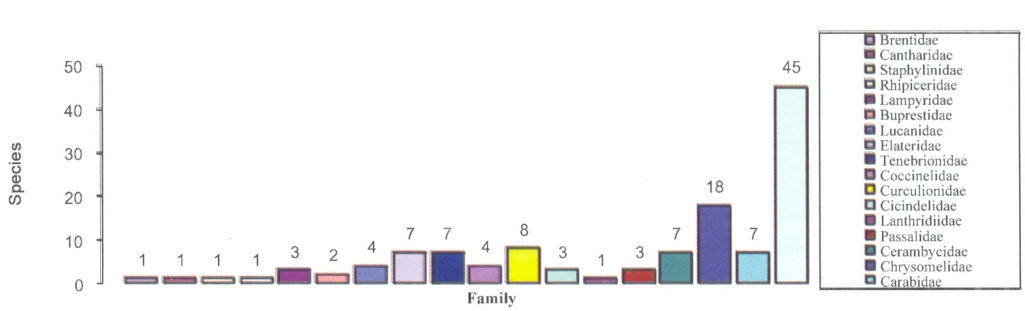

Figure 3. Bar chart below shows the number of species for each family of beetle at Kubah National Park

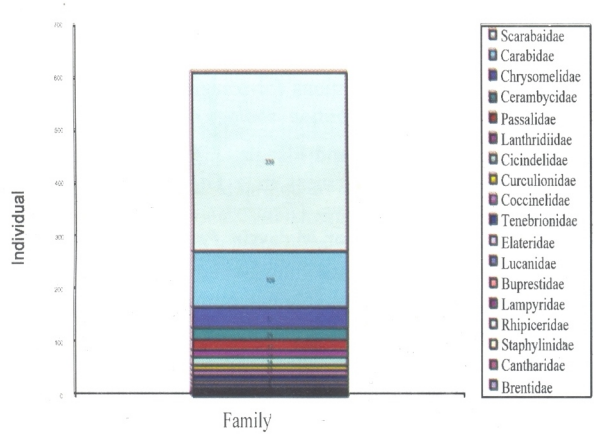

Figure 4. Relative abundance of beetle family at Kubah National Park.

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Ethological patterns of Silvered Leaf Langur (*Presbytis cristata*) at Bako National Park

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Ethology is a study of whole patterns of animal behavior under natural conditions, in ways that emphasized the function and the evolutionary history of the patterns (Eisner and Wilson, 1975). The comparisons of proportion time that animal like silvered leaf langur spend in different activities are important to assess inter and intraspecific behavioral differences and may be used to identify the adaptive nature of variability in temporal patterning of activities (Robinson, 1986). The activity pattern, time budget and diet of silvered leaf langur (Presbytis cristata) were studied at Telok Assam, Bako National Park, Sarawak. This study was done on two separate sampling occasions: first sampling was on 28 August 2006 until 1 September 2006 and second sampling was on 23 November 2006 until 29 November 2006.

Figure 1: Silvered Leaf Langur (*Presbytis cristata*) at Bako National Park

Bako National Park was gazetted in 1957 and is the oldest national park in Sarawak, located at the Muara Tebas Peninsula, about 30 km north of Kuching city, southwest Sarawak. Behaviour of P. cristata was recorded by using the group scanning method and focal sampling. During the twelve days of field sampling from 0600 hours until 1800 hours, 3334 minutes were spent observing the behaviour of P. cristata. The result indicated that 1145 minutes (34.34%) of the half day was spent moving, 990 minutes (29.69%) was spent resting, 655 minutes (19.65%) was spent feeding, 395 minutes (11.85%) was spent socializing and 149 minutes (4.47%) was spent foraging.

Physically, *P. cristata* has an average body mass of 6.6 kilograms for adult males and 5.7 kilograms for adult females (Fleagle, 1999). Its appearance is entirely dark, metallic grey and their face are black or dark grey in color (Furuya, 1961), and their infants are bright orange, with the face, hands, and feet being white (Bernstein, 1968; Payne et al., 1998). Socially, P. cristata has a unimale social structure. The group is composed of single adult male, several adult females, and immature individuals (Wolf and Fleagle, 1977). Males will live solitarily before finding a group of its own (Bennett and Davies, 1994). The groups are highly territorial and will act aggressively towards other individuals (Bernstein, 1968). Males will keep other group males out of the range of the group (Bernstein, 1968; Furuya, 1961). The observations from this study indicated that the total population of silvered leaf langur in the Telok Assam trail is approximately 30, including six females and their infants.

P. cristata selected Otak Udang (*Buchanaia* sp) tree at the boat shed, Ubah tree (*Syzgium* sp.) and Otak Udang tree behind the Park Headquarters as their sleeping sites. Their observed diet composed of flowers, young leaves, buds, and barks of Baru Laut tree (Hibiscus tiliaceus), young leaves of Otak Udang tree (*Buchanania* sp.), young leaves of Leban tree (*Vitex pubescens*), leaves and fruits of Pandan (*Pandanus* sp.), and young leaves of Paku Kubok (*Nephrolepis biserrata*). It is hoped that the park management should nurture and enhanced these tree species, as a continuous food resource, for this natural tourist attraction at Bako National Park. P. cristata has a large observed home range, as they moved to feed on the food resources in the park, besides the area around the Telok Assam.

Acknowledgement

We would like to acknowledge the Forest Department, Sarawak Forest Corporation and Bako National Park for the permit and permission to work at Bako. Our special gratitude also goes our colleagues and staff from Universiti Malaysia Sarawak (UNIMAS) for all their help, support and cooperation during the duration of this study.

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The Utility of Microsatellite Markers in the Preliminary Study of the Genus *Cynopterus* in Peninsular Malaysia, Sabah and Sarawak Regions.

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Bats from genus Cynopterus is one of the widely distributed bats that can be found in the Indo-Malayan region. Recent studies using mitochondrial DNA data suggests that there is a cryptic species within *C. brachyotis* in Peninsular Malaysia up to southern Thailand. Based on these studies, forearm length is the only external morphological measurement that can be used to differentiate *C. brachyotis* and the cryptic species. However, hybridization might happen in the forest fringe where both species can be found. In this study, *C. brachyotis* and *C. horsfieldii* in Borneo were included and examined using four selected existing microsatellite markers. However, in this preliminary study only basic information derived from the microsatellite data of *C. brachyotis* (with its cryptic species), *C. sphinx*, and *C. horsfieldii* were produced.

Table 2: Genotypic disequilibrium with P-value for each locus pair across all populations using Fisher's method.

Locus pair	χ^2	df	P-value
CSP1 vs CSP3	10.436	8	0.236
CSP1 vs CSP4	15.589	8	0.049*
CSP3 vs CSP4	4.421	8	0.817
CSP1 vs CSP7	17.988	8	0.021*
CSP3 vs CSP7	16.156	8	0.040*
CSP4 vs CSP7	3.782	8	0.876

* indicates significant, χ^2 = chi square, df = degree of freedom

A total of 93 alleles were generated from 54 samples of large form *C. brachyotis*, 11 samples of small form *C. brachyotis* (cryptic species), 30 samples of *C. sphinx* and 42 samples of *C. horsfieldii*. Generally most of the populations are deviate from the Hardy-Weinberg equilibrium. Genotypic disequilibrium across populations (P-value < 0.05) indicated that there are linkages between the loci CSP1 versus CSP4 (P-value = 0.049), CSP1 versus CSP7 (P-value = 0.021) and CSP3 versus CSP7 (P-value = 0.040). This is most notably observed in locus pairs of *C. brachyotis* (CSP1 versus CSP4) and *C. horsfieldii* (CSP1 versus CSP7 and CSP3 versus CSP7).

In this study, we are able to generate the basic information for the cynopteran fruit bats from Malaysian Borneo. We also suggest an extension of the study using more microsatellite loci and a larger sample size with representatives from Kalimantan, Sulawesi and the Philippines as this would provide information on the wide spread *Cynopterus* populations in this region.

Cynopterus brachyotis (Photo by Chong Yee Ling)

Cynopterus horsefieldii (Photo by Chong Yee Ling)

Genetic and Morphological Species Identifications of the Malaysian Roundleaf Bats (Chiroptera: Hipposideridae)

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Approximately 113 bat species have disjunct populations on Borneo and Peninsular Malaysia, which are separated by about 600 km South China Sea. This magnitude of geographic isolation created by inundation and expansion of sea level within this region, that occurred in several stages starting from 2 MYA until the last glaciations about 10,000 years ago may have resulted in reduced gene flow between populations by enough time for speciation to occur. To assess genetic diversity in these bats and to compare genetic and morphological species identifications, we generated and phylogenetically analyzed 404 base pairs of the mitochondrial cytochrome-*b* (cyto *b*) gene. Herein, we focus on the family Hipposideridae and compare two species (*Hipposideros cervinus* and *H. bicolor*) to estimate intra- and interspecific genetic variation.

Within *H. cervinus* complex, we identified eleven haplotypes, separated by <2% in cyt-*b* variation, indicating that these populations have not been isolated for a sufficient amount of time to speciate. Field identifications of individuals within this complex suggested at least two species were present (Figure 1). In contrast to *H. cervinus*, our Borneo and Peninsular samples of *H. bicolor* were separated by genetic distance values averaging 4-9%, indicating that these populations have been genetically isolated (Figure 2). However, this isolation was not reflected in the external morphology of specimens. Additionally, there were no shared haplotypes between Borneon and Peninsular Malaysian populations of either *H. cervinus* or *H. bicolor*. Morphological species identifications are necessary to assess bat diversity in the field. However, the results from the family Hipposideridae

Figure 1: Colour variation within *H. cervinus* that is not reflected in genetic divergence.

Photo by Dr. Robert J. Baker (Texas Tech University) Park

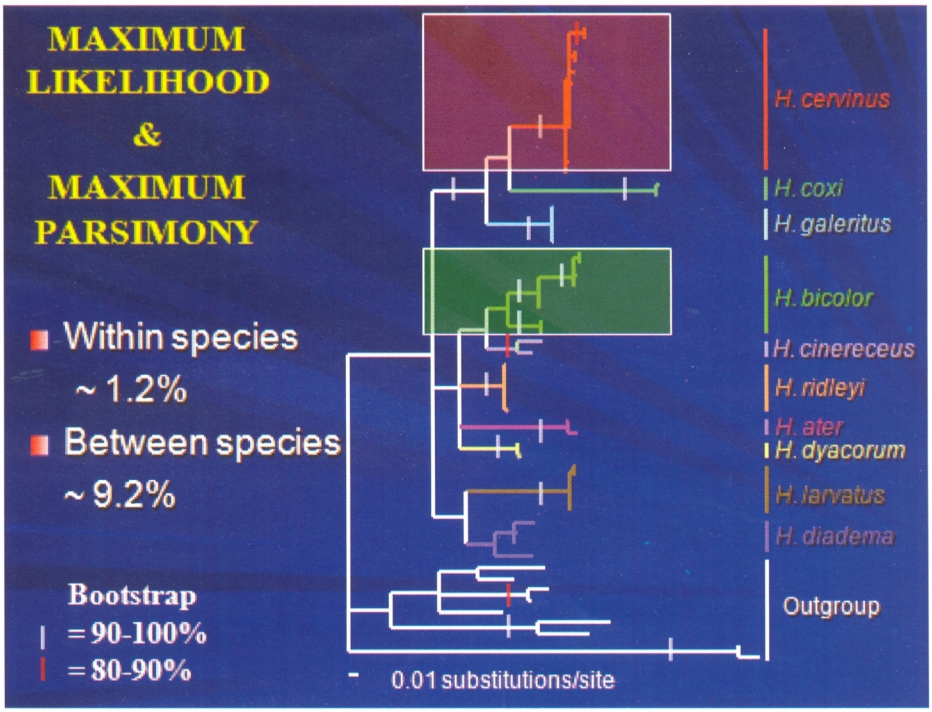

Figure 2: Maximum Likelihood topology for 10 Hipposideros species of Malaysia. Topology includes boostrap value (marked with white and red lines) from Maximum Likelihood and Maximum Parsimony. Average genetic divergence within clade (within species) is 1.2% and between clade (between species) is 9.2%. *H. cervinus* clade (in red) shows less than 2% variation despite their distinct colour variation within them. *H. bicolor* clade (in green) divergence of 4 to 9% indicating more than one species is currently present within this species complex. This has been illustrated by Kingston *et al.* (2001), that correspond to two different echolocation call (131 khz and 142 khz) within two different peninsular Malaysia form with 6% genetic divergence. This study recognized another clade within *H. bicolor* complex from Borneo that has 5 to 10% divergence compared to the other two forms from Peninsular Malaysia. Individuals from Borneo were also recorded with 136 khz echolocation calls (Hall personal communication) that might be significant in the ecological context to differentiate within all three forms of *H. bicolor*.

indicate that identifying species using only external morphology could result in an over - or underestimation of bat diversity. We propose that genetic data should be used first to identify species and then used with detail morphological data to develop greater accuracy of faunal studies. [*Presented by **Faisal A. Anwarali**, in the **First International South East Asia Bat Conference** organized jointly by Prince Songkhla University, Texas Tech University and The Harrison Institute at Club Andaman Resort Beach Hotel, Phuket, Thailand.]

New Gonyautoxin Analogue Isolated from Toxic Dinoflagellate (Dinophyceae) *Alexandrium Minimum*

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Toxicity of five clonal cultures of dinoflagellate, Alexandrium minutum (Figure 1) established from aquacultures pond, located in Hai Phong, Northern Vietnam were analysed using HPLC according to Oshima (1995), with isocratic, post-column derivatization method on a JASCO HPLC system (JASCO, Japan). The species composed of predominantly GTX4 (>70%), with GTX1 and neoSTX as minor components (Figure 2). GTX3 and GTX2 were detected in trace amount. In addition, a new gonyautoxin was found eluting between GTX4 and GTX1 in HPLC. The peak disappeared under non-oxidizing HPLC condition but unchanged either after treated with 0.05 M ammonium phosphate/ 10 % mercaptoethanol or 0.1 N HCl hydrolysis. For LCMS/MS, mass cultures of A. minutum were carried out in twenty 10L culture vessels with mild aeration. Culture at late exponential phase was harvested extracted in 0.5 M acetic acid (AcOH). The extract was treated with 0.05 M ammonium phosphate/ 10 % (v/v) mercaptoethanol (ME) and boiled for 10 minutes to remove the sulformoyl moiety of GTXs according to Sato and Kodama (2003). Conversion of GTX1, 2, 3, and 4 to STXs (STX and NEO) was confirmed by HPLC analysis. The extract was then purified using a Bio-Gel P-2 column (fine; 15 mm ¥ 450 mm) (BioRad, Hercules, CA, USA) and eluted with 0.2 M AcOH at a flow rate of 0.5 mL min-1. LCMS/MS ion scanning of the purified compound showed a parental ion of [M+H]+ at m/z 396, [M-SO3]+ at m/z 316, and [M-SO4]+ at m/z 298. The derivative was identified as deoxy-GTX4-12ol.

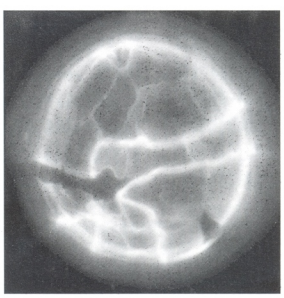

Figure 1: *Alexandrium minutum* stained with calcofluor white and observed under epi-fluorescence microscope.

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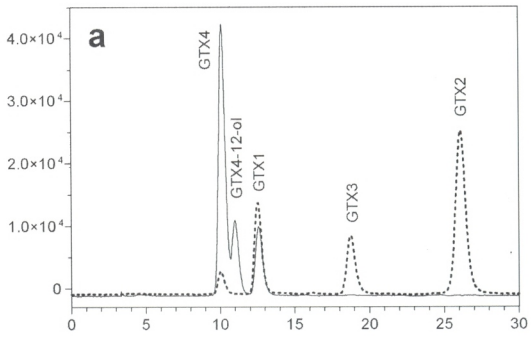

Figure 2: Toxin composition of *Alexandrium minutum* with GTX4-12ol located between GTX4 and GTX1. Toxin standard was shown in dash line.

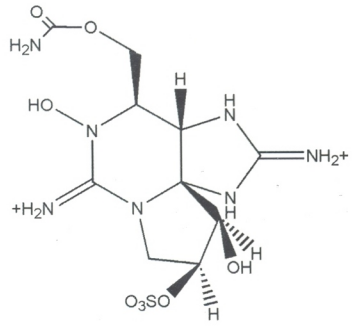

Figure 3: Deoxy-GTX4-12ol

Sexing of Monomorphic Spiderhunters (Family: *Nectariniidae*) using Logistic Regression Analysis

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Spiderhunters (Family: Nectariniidae) are monomorphic birds, i.e., having similar external morphological characters and the same feather colorations, and thus difficult to determine whether they are male or female, through observation (Merry, 1999; Møller, 1994). Sexing or sexual dimorphism of spiderhunters was investigated, based on measurements of the specimens' seven external morphological characters (kept in the Sarawak Museum), and analyzed using logistic regression analyses. The dependent variable of logistic regression is binary or dichotomous, and can be represented by a binary indicator variable, taking the values of 0 and 1 (Figure 1). Logistic regression is either the simple model (1 independent variable) or multivariate logistic model (two or more independent variables), (Hosmer and Lemeshow, 2005; Collett, 1991; Brannick, 2005).

A total of 8 species of spiderhunters, with 181 individuals (98 males, 83 females) were examined. Four prediction models were found with their respective parameters: bill length (BL) for little spiderhunter (*Arachnothera longirostra*), and wing length (WL) for yellow-eared spiderhunter (*A. chrysogenys*), long-billed spiderhunter (*A. robusta*) and grey-breasted spiderhunter (*A. modesta*), respectively. However, the other species including thick-billed spiderhunter (*A. crassirostris*), spectacled spiderhunter (*A. flavigaster*), streaky-breasted spiderhunter (*A. affinis*) and whitehead's spiderhunter (*A. juliae*) showed no significant differences of gender, in their external morphological characteristics.

The parameters that were found to significantly differentiate male and female of spiderhunters were the bill length (BL) for little spiderhunter, and wing length (WL) for yellow-eared spiderhunter, long-billed spiderhunter and grey-breasted spiderhunter respectively. The respective logistic equations were:

1. Little spiderhunter: $Y = 20.942 - 0.71(BL)$
2. Yellow-eared spiderhunter: $Y = 55.05 - 0.6997(WL)$
3. Long-billed spiderhunter: $Y = 19.114 - 0.2273(WL)$
4. Grey-breasted spiderhunter: $Y = 18.676 - 0.239(WL)$

Deviance method was used to examine the goodness-of-fit for these equations. All 4 equations obtained a p-value of >0.05, depicting that there were no evidence of a lack-of-fit and therefore, these model were appropriate. Overall, the percentage of correct prediction is 81.36% 91.38%, 85.71% and 80.0%, respectively for little spiderhunter, yellow-eared spiderhunter, long-billed spiderhunter and grey-breasted spiderhunter. Results also showed that males are relatively larger than females based on the 7 selected external morphological characters.

Generally, spiderhunters showed that males are relatively larger than females in their selected external morphological characters, which may have resulted from natural selection and/or sexual selection.

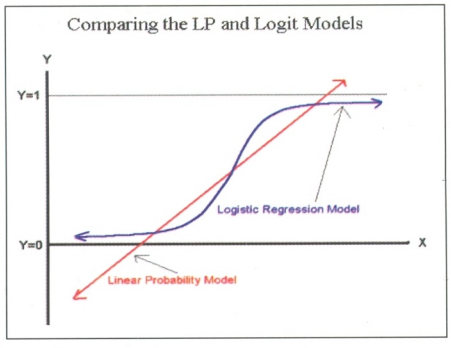

Figure 1: Comparison between the logistic regression model and linear model. (Source: Whitehead, 2005).

Acknowledgement

We would like to acknowledge Dr. Charles Leh from Sarawak Museum for permission to examine the spiderhunter specimens, and colleagues and staff from Universiti Malaysia Sarawak (UNIMAS) for all their help, support and cooperation during the duration of this study.

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Figure 2: Little Spiderhunter (*Arachnothera longirostra*)

Figure 3: Sarawak Museum samples of Spiderhunters (Family: Nectariniidae)

Using Multivariate Analyses to better distinquish Morphometric Variations between Squirrels (genus *Callosciurus*)

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The genus *Callosciurus* consisted of five species, namely *Callosciurus baluensis*, *C. prevostii*, *C. orestes*, *C. notatus* and *C. adamsi* (Payne *et al.*, 1998) This study described the morphometric variations within this genus using existing specimen and data from Sarawak. Currently, there is no study on the phylogeny of genus *Callosciurus* based on morphometrics cluster analysis in Borneo. Morphometric phylogeny using large data set characters would be able to explain the morphological variation and based on these variations the relationship among the species can be further explained (Jayarajl *et al.*, 2006). Field sampling were conducted at Bako National Park, Satang Island and Batang Ai National Park. Samples from Zoological Museum at Universiti Malaysia Sarawak and Sarawak Museum were also utilised for this study. A total of 33 specimens were examined based on 16 morphometrics characteristic (external body, skull and dental).

All 16 parameters were analyzed using the discriminant function analysis (DFA) and canonical variate analysis (CVA). Multivariate morphometrics also applies quantitative methods to discover structural interrelationships of multiple measurements, based on within and between groups using real biological data set (Oxnard, 2000, Afifi and Clark, 1984; Bernstein, 1988). Discriminant function is placement of unknown specimens into known groups to increase the discrimination between groups based on a set of measurements. For canonical variate analysis, graphical ordination or visualization is used to recognize relationships between each group in multidimensional space (Reyment *et al.*, 1984). Cluster analysis is a worthwhile alternative to DNA sequencing analysis (Jayaraj *et al.*, 2006), given a sample and grouping them according to their "similar" characters in the same class (Manly, 1994). Cluster analysis has a wide variety of procedures to create a classification with observed "closer similarities" of each group (Dunteman, 1989).

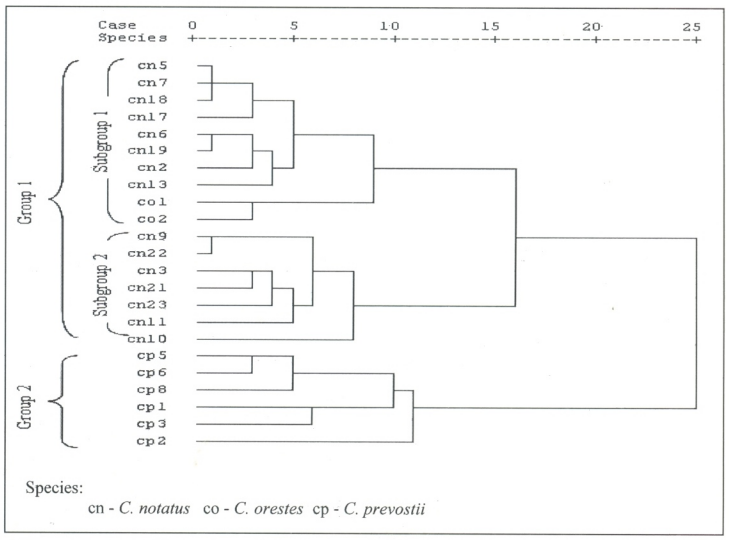

Figure 2: Cluster analysis of *C. notatus*, *C. orestes* and *C. prevostii*.

The highest characters of the two functions from DFA (Discriminant Function Analysis): Function 1 was dietary length (DL), and for Function 2, were, tail (T), zygomatic width (ZW), bulla length (BL), cranial width (CW) and condoylobasal length (CBL). Cluster analysis was used to confirm the grouping of all samples into two groups, with the first group was *C. notatus* and *C. orestes* and the second group was *C. prevostii*.

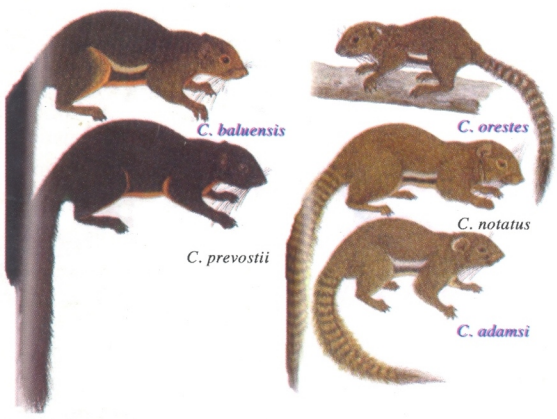

Figure 2: Species under Genus Callosciurus (Source: Payne *et al.* 998)

Unfortunately, the resolution of these analyses was still not good enough to fully address the separation of the 3 species. It was noted that individuals of *C. orestes* were mixed with the *C. notatus* in a subgroup. There was very reason to believe that the 2 samples of *C. orestes* might have been wrongly identified and that they were actually 2 samples of *C. notatus*.. The formation of these 2 sub-groups of *C. notatus* was also a mistery, which may be an indication of a sub-species or a cryptic species. A further in-depth study using molecular genetic tools is going to be necessary to address these further queries. Further studies should also include all five know species of Genus *Callosciurus*, while recognizing the sample collection of these species will be a daunting task.

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